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AB0063 THE PROTECTIVE EFFECT OF PFTα ON ALCOHOL-INDUCED OSTEONECROSIS OF THE FEMORAL HEAD

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Background: Epidemiologic studies have shown that alcohol plays a pivotal role in the development of osteonecrosis of the femoral head (ONFH). However, few studies have discussed the pathogenesis of or interventions for alcohol-induced ONFH.
Objectives: The aim of this study was to explore the underlying mechanism of alcohol-induced ONFH and the protective effect of pifithrin-α (PFTα).
Results: Through a series of *in vitro* assessments, we found that ethanol treatment significantly activated p53, suppressed Wnt/β-catenin signaling and inhibited osteogenic-related proteins, including runt-related transcription factor 2 (RUNX2), osteocalcin (OCN), osteopontin (OPN) and collagen I (COL1). Furthermore, by separating the cytoplasmic and nuclear proteins, we found that ethanol inhibited osteogenesisby impairing the accumulation of β-cateninin both the cytoplasm and nucleus in human bone mesenchymal stem cells (hBMSCs), which resulted from activating glycogen synthase kinase-3β (GSK-3β). In this *in vivo* study, we established alcohol-induced ONFH in rats and investigated the protective effect of PFTα. Micro-CT, hematoxylin & eosin (H&E) staining, immunohistochemical analyses, immunofluorescence staining, TUNEL staining, and fluorochrome labeling were performed to reveal the PFTα-induced therapeutic effects. H&E findings combined with TUNEL, caspase-3-cleaved immunohistochemical staining, and micro-CT images revealed obvious ONFH in the alcohol-administered rats, whereas significantly less osteonecrosis developed in the rats injected with PFTα. As the initiator of osteogenesis, RUNX2 and its downstream targets OCN, OPN, COL1 were immunostained in the femoral heads. These results indicated that those osteogenic-related proteins were significantly decreased in the alcohol-administered rats, whereas these results were reversed in the PFTα-injected rats. Fluorochrome labeling showed a similar result in that alcohol significantly reduced the osteogenic activity in the rat femoral head, which was blocked by the injection of PFTα.
Conclusions: Pifithrin-α, a p53 inhibitor, was able to block the ethanol-triggered activation of p53 in hBMSCs and alcohol-induced ONFH in a rat model. Its antagonistic effect against ethanol's effect on hBMSCs could be a clinical strategy to prevent the development of alcohol-induced ONFH.
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AB0064 ADIPOSE STROMAL CELLS EXERT SPECIFIC EFFECTS ON OSTEOARTHRITIC SYNOVIAL MACROPHAGES

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Background: Osteoarthritis (OA) is the most common joint disease and the major cause of pain and disability in the aging population [1]. Adipose stromal cells (ASC) are promising candidate for cell therapy in OA, because they have immunomodulatory, trophic and differentiation capacities [2,3]. Synovial inflammation is accepted as important OA feature for the symptoms and disease progression [4]. Synovial tissue is mainly composed of synovial fibroblasts (SF), macrophages (SM) and a low percentage of other cell types [5].
Objectives: Aim of the study was to analyze the effects of adipose stromal cells in co-culture with SF and SM.
Methods: GMP clinical grade ASC were isolated from subcutaneous adipose tissue. Synoviocytes were isolated from synovial tissue of OA patients undergoing total joint replacement. Synovial cells at passage 1 and 5 were analyzed for: 1. different phenotypical markers by flow cytometric analysis, 2. inflammatory factors by multiplex immunoassay, 3.anabolic and degradative factors by qRT-PCR. Both p.1 (mix of SF and SM) and p.5 (only SF) synovial cells, as different cell models, were co-cultured with adipose stem cells (ASC) to define their effects. Furthermore macrophages type 1 (M1) were isolated and co-cultured with ASC.
Results: Synoviocytes at passage 1 were positive to typical markers of SM (CD14,CD16,CD68, CD80,CD163) and SF (CD55,CD73,CD90,CD105,CD106), whereas at passage 5 were positive only to SF markers and showed a higher percentage of CD55 and CD106. At p.1 synovial cells released a significantly higher amount of all inflammatory (IL6,CXCL8,CCL2,CCL3,CCL5) and anabolic (IL10) factors than those at p.5. Moreover, p.1 synovial cells expressed also higher amount of some degradative factors (MMP13, S100A8, S100A9) than p.5 synovial cells. Co-culture experiments showed that the amount of SM in p.1 synovial cells specifically orchestrate the up or down-modulation of some inflammatory (IL6,CXCL8,CCL2,CCL3,CCL5) and degradative factors (ADAMTS5,MMP13, S100A8,S100A9) analyzed. Interestingly, p.5 synovial cells induced all factors analyzed, except CCL5. Finally, we demonstrated that ASC effects were strictly dependent by M1, that decreased the release of typical macrophages cytokines (IL1β, IL6, TNFα and CCL3/MIP1α) and that ASC effects are responsible for the switching by M1 like inflammatory macrophages to M2 like phenotype mainly due to PGE2 involvement.
Conclusions: These data demonstrate that the GMP-ASC effects on OA synovial inflammation are strictly dependent by macrophages, that orchestrate the switching activated-M1 inflammatory macrophages to a M2-like phenotype, mainly through PGE2
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AB0065 2-CARBA-CYCLIC PHOSPHATIDIC ACID SUPPRESSES EXPRESSION OF CARTILAGE DEGRADING ENZYMES SUCH AS MMP-13 IN INFLAMMATORY SYNOVIAL FIBROBLASTS AND ARTICULAR CHONDROCYTES INDUCED BY IL-1 BETA AND/OR TNF ALFA

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Background: Cyclic phosphatidic acid (cPA) is one of bioactive lipid, has been implicated as a mediator of various biological effects including inhibitory effects of proliferation, invasion and metastasis of cancer cells. cPA is naturally occurring mediator even exists in human serum. 2-carba-cPA (2ccPA) is structurally modified formula of cPA and has shown improved bioactivity. We have previously confirmed that 2ccPA stimulated HAS-2 production on human osteoarthritic chondrocytes and synovial fibroblasts (SFs) in vitro. Furthermore, intra-articular administration of 2ccPA has shown its suppressing effect of pain, swelling, and articular cartilage destruction in rabbit experimental osteoarthritis (OA). We have shown that 2ccPA might had direct inhibitory effect of cartilage degrading enzymes on rheumatoid synovial fibroblasts (SFs) in vitro. Inflammatory arthritis such as rheumatoid arthritis (RA) and early stage of OA involves synovial inflammation and subsequent production of cartilage degrading enzymes also from chondrocytes. 2ccPA may be possible another therapeutic option for degenerative arthritis.
Objectives: The aim of this study was to evaluate the direct effects of 2ccPA on cartilage matrix degrading enzymes using SFs and articular chondrocytes under influence of inflammatory cytokines.
Methods: In vitro studies were performed using SFs and chondrocytes obtained from arthritis patients (RA and OA) at joint replacement surgery. First, 2ccPA 0–25 μM was added to cell cultures and effects of 2ccPA on ADAMTS-4, -5, MMP-3, 9, -13 expression was assessed by real time PCR using specific primers to corresponding genes. Beta-actin was used as endogenous expression control for PCR. As we confirmed that 2ccPA had dose-dependent inhibitory effects on expression of above enzymes, the second experiment was performed. SFs or chondrocytes were pre-cultured with IL-1β (1 ng/ml) and/or TNF-α (10 ng/ml) for 24 hours, then added 10 μM 2ccPA to study attenuated effect of 2ccPA on synthesis of above cartilage matrix degrading enzymes. Newly synthesized MMP-3, -13 from SFs or chondrocytes in cultured media after 24 hours of 2ccPA addition were measured by sandwich ELISA.
Results: 2ccPA itself repressed cartilage degrading enzymes, ADAMTS-4, ADAMTS-5, MMP-3, MMP-9 and MMP-13 expression in both SFs and chondrocytes was all repressed by low dose of 2ccPA. Even after cells had been stimulated by cytokines, 10 μM 2ccPA repressed expression of cartilage degenerating enzymes in SFs or chondrocytes. Expression of MMP-13 were repressed more in chondrocytes by 2ccPA. ELISA results also confirmed the inhibitory effect of 2ccPA on MMP-13 production in RA SFs (n=5) by 52% or in RA chondrocytes (n=3) by 43%. In OA, MMP-13 production was reduced by 31% in OA SFs (n=6) and 34% in OA chondrocytes (n=4). However, not significant reduction of MMP-3 in both SFs or chondrocytes. MMP-9 expression by ELISA was under the detectable limit.

Conclusions: The in vitro results confirmed that 2ccPA had suppressing effect of cartilage degrading enzymes expression on SFs and chondrocytes, supports the hypothesis that 2ccPA might have played direct role to suppress inflammation and also protect articular cartilage in arthritic condition.
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AB0066 HUMAN MULTIPOTENTIAL STROMAL CELLS EXPRESS LOW SURFACE LEVELS OF PRO-INFLAMMATORY CYTOKINE RECEPTORS IN BONE HEALING DEFECTS

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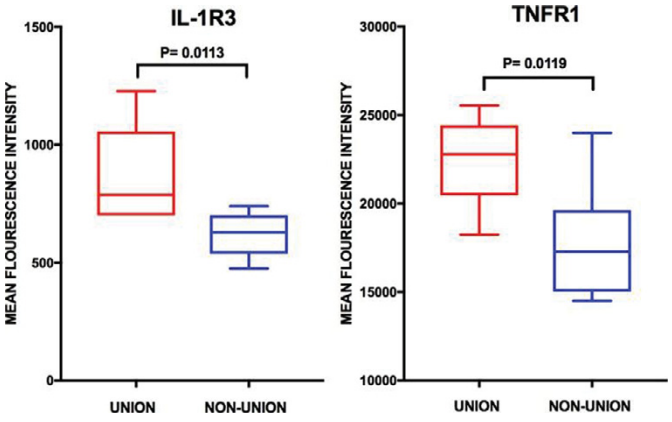
Background: Osteoimmunology is an evolving field where the multipotential stromal cells (MSCs) can be considered as an important player linking immune response with bone generation. A group of pro-inflammatory cytokines including IFN-γ, TNF-α, IL-17 and IL-1, has been proven to have a licensing effect on MSCs promoting the immunomodulatory activities of MSCs (1). Importantly, these cytokines can regulate the osteogenic differentiation capability of MSCs and in particular, IL-1 and IL-17 can enhance the MSC osteogenesis as shown in previous in vitro studies (2,3). However, little is known about the role of these cytokine-MSC interactions in the bone-related diseases in humans.
Objectives: The main focus of this study was to assess if the immune-dependent licensing process of MSCs could be involved in defective bone regeneration.
Methods: We used samples of bone marrow aspirates (n=15) from two groups of traumatic bone fracture patients; normal union and non-union. Bone marrow MSCs were analyzed for the surface expression of the receptors of the pro-inflammatory licensing cytokines using flowcytometry-optimized panels. Additionally, a comparison of the cytokine effect on the proliferation of cultured MSCs was compared between normal union and non-union groups using the cell proliferation XTT test.
Results: Interestingly, there were significant lower expression levels of IL-1 receptors 1 and 3 (IL-1R1 and IL-1R3) on non-union MSCs compared to normal-union MSCs (p=0.0478 and p=0.0113 respectively). Furthermore, the surface levels of TNF-αR1 (CD120a) were significantly lesser on non-union MSCs (p=0.0119). There was a clear trend of reduced expression of IL-17 receptors (CD217) on the surface of non-union MSCs, but it was not statistically significant compared to normal-union (p=0.0726). The XTT data showed a significant less proliferation index for IL-1-treated non-union MSCs compared to normal-union MSCs (p=0.0446). Also, a consistent trend of lower proliferation index of non-union MSCs was detected when these cells were treated by IFN-γ, TNF-α or IL-17.



Conclusions: Together, the lower levels of the pro-inflammatory cytokine receptors indicated a possible mechanism for a defective response of non-union MSCs to the inflammatory signals (particularly IL-1). Further understating of the impact of immune-MSC interactions on human bone healing and regeneration will help to develop new therapies for musculoskeletal diseases involving osteolytic lesions.
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AB0067 INFLUENCE OF ADIPOKINES ON DIFFERENTIATION OF SPONGIOSA-DERIVED MESENCHYMAL STROMAL CELLS FROM OSTEOPOROTIC AND OSTEOARTHRITIS PATIENTS

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Background: Osteoporosis (OP) and osteoarthritis (OA) are two common age-related disorders leading to chronic pain and disability in elderly people. Age-related bone loss and articular cartilage damage are associated with increased bone marrow adiposity due to a possible shift of osteogenic differentiation towards adipogenic differentiation of bone marrow mesenchymal stem cells (MSC). The differentiation of MSC into adipocytes or osteoblasts is an important determinant of bone structural integrity. Adipose tissue is an metabolically active tissue. Therefore adipocyte-derived factors -adipokines- might influence differentiation of bone marrow-derived MSC.
Objectives: The role of fat-bone interactions in the pathogenesis of OP is poorly understood. Therefore, we analyzed the presence of distinct adipokines (visfatin, resistin and leptin) in the bone marrow cavity and their effects on MSC differentiation.
Methods: Spongiosa from femoral heads was collected (hip replacement surgery of OA patients or after osteoporotic femoral neck fracture). MSC were cultured in adipogenic and osteogenic media with/without adipokines. For the transfer and differentiation of MSC on cancellous bone, bone fragments were purified and sterilized. mRNA expression of adipokines, bone marker genes, TIMPs and MMPs of stimulated MSC and bone samples were evaluated by realtime PCR. Matrix mineralization was assayed using Alizarin red S staining. Proinflammatory factors were measured by ELISA.
Results: Visfatin and leptin levels were increased in OP bone vs. non-osteoporotic bone (n=14). In contrast to leptin and resistin, visfatin induced the secretion of proinflammatory factors (IL-6, IL-8, MCP-1) during both, osteogenic and adipogenic differentiation. Visfatin significantly increased the matrix mineralization and downregulated collagen type 1-expression (e.g. d21: -4.6-fold) in osteogenic differentiated cells. Visfatin also reduced the expression of MMP2, MMP13, RunX2, TIMP1 and TIMP2 (e.g. d21: -2.4-fold/-3.18-fold/-5.85-fold/-3.2-fold/-4.3 fold respectively) during osteogenic differentiation, but not leptin and resistin. In contrast to osteogenesis, visfatin significantly induced MMP13 expression (e.g. d21: 104-fold) during adipogenic differentiation under standard cell culture conditions. However, visfatin-induced MMP13-expression was markedly reduced during differentiation on purified autologous cancellous bone.
Conclusions: Visfatin and leptin levels were elevated in osteoporotic bone tissue. Therefore, the visfatin-mediated increase of matrix mineralization and reduction of collagen type 1 expression might lead to enhanced bone fragility and contribute to the pathogenesis of OP. Visfatin induced release of proinflammatory cytokines and dysregulated expression of MMPs and TIMPs during osteogenic and adipogenic MSC-differentiation might influence bone turnover specifically at the adipose tissue/bone interface.
Disclosure of Interest: None declared
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AB0068 HYPOXIA AND RHEUMATOID PHENOTYPE DECREASE THE CAPACITY OF SYNOVIAL FIBROBLASTS TO SUPPRESS T HELPER CELL PROLIFERATION THROUGH IDO1-MEDIATED TRYPTOPHAN CATABOLISM

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Background: The pathogenesis of rheumatoid arthritis (RA) is linked to functional changes in synovial fibroblasts (SF) and local infiltration of T lymphocytes. Increased synovial inflammation is also associated with a hypoxic joint microenvironment. Oxygen levels in the joints of RA patients are significantly decreased compared to those of osteoarthritis (OA) patients with values of about 22.5mmHg corresponding to ambient oxygen tensions of 3.2%. So far, little is known about the effects of hypoxia on the interaction between fibroblasts and T lymphocytes and its implications on the pathophysiology of RA.
Objectives: The aim of this study was to compare the influence of SF from RA versus OA patients on T helper (Th) cell responses both under normoxic and hypoxic conditions.
Methods: SF were isolated from synovectomy tissues of OA or RA patients, Th cells were isolated from peripheral blood of RA patients or healthy donors. Cell cultures were performed under normoxic or hypoxic (3% O_2) conditions. Th cell proliferation was determined by PKH26 labelling and flow cytometry. Cytokine secretion was quantified by ELISA. Indoleamine 2,3-dioxygenase 1